Chemical and Biological Determination of Coumaphos Concentration in Cattle-Dipping Vats

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Chemical and Biological Determination of Coumaphos Concentration in Cattle-Dipping Vats

By R. O. Drummond[1] and W. W. Utterback[2]

ABSTRACT

Samples from 10 regularly used vats and 4 disused but charged vats operated by the U.S. Animal and Plant Health Inspection Service and the Texas Animal Health Commission in south Texas were analyzed by a chemical technique and by bioassay with engorged female *Boophilus microplus* (Canestrini) ticks to determine concentration of coumaphos [Co-Ral; *O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) *O*,*O*-diethyl phosphorothioate]. The bioassay technique adequately estimated coumaphos concentration (acaricidal activity) in a sample, and there was a significant correlation between this determination and the chemical analysis, although the bioassay values were about 75 percent of values determined by chemical analysis. When the regularly used vats were replenished with water and coumaphos (25 percent wettable powder) as mandated by the policy of the Cattle Fever Tick Eradication Program, the coumaphos remained acaricidally active through 1 year of monitoring. In disused vats, there was no loss in acaricidal activity for 2 years, and the chemical analysis accurately measured that activity. Comparison of coumaphos concentrations determined by chemical analysis of samples taken before and after dipping indicated that there was considerable variation in the effectiveness of vat-mixing methods. The amount of coumaphos wettable powder prescribed for initial charge and replenishment (0.165 percent, or 5.5 pounds per 100 gallons of water) was sufficient to kill susceptible, engorged *B. microplus* and *Boophilus annulatus* (Say) females because the concentration equaled or exceeded the calculated LC_{99} for the ticks. Index terms: acaricides, *Boophilus annulatus* (Say), *Boophilus microplus* (Canestrini), cattle-dipping vats, Cattle Fever Tick Eradication Program, coumaphos, pesticides, test methods, ticks.

INTRODUCTION

The presence of *Boophilus microplus* (Canestrini), the southern cattle tick, and *B. annulatus* (Say), the cattle tick, in Mexico constitutes a continual threat to the U.S. cattle industry. These species were eradicated from about 1,813,000 square kilometers of the United States after a 40-year campaign (Graham and Hourrigan 1977) and are prevented from reintroduction from Mexico by the Cattle Fever Tick Eradication Program of the U.S. Animal and Plant Health Inspection Service and the Texas Animal Health Commission. This program includes the treatment of cattle imported from Mexico and the maintenance of a buffer zone along the Rio Grande from Brownsville

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to Del Rio, Tex. Cattle may be moved from this zone only after examination by State or Federal inspectors and treatment with an approved acaricide. Also, tick outbreaks outside the buffer zone are eliminated by systematic dipping of cattle or by removing cattle from infested premises for specific periods of time.

One acaricide approved for use in the program is coumaphos [Co-Ral; *O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) *O*,*O*-diethyl phosphorothioate] as a 25-percent wettable powder (WP) at a vat concentration of 0.125 to 0.175 percent. Current policy calls for the charging of official dipping vats with 5.5 pounds of 25-percent-WP coumaphos per 100 gallons of water; this rate creates a concentration of 0.165 percent coumaphos, and replenishment is at the same rate. Vats must be replenished whenever the volume of fluid falls below seven-eighths of the full level, and must be emptied, cleaned, and recharged when any one of the following occurs: (1) 120 days have elapsed since the vat was initially charged, (2) two animals per gallon of vat capacity have been dipped, or (3) 10 percent or more by volume of sediment and debris have accumulated in the vat fluid. These procedures were established to insure a sufficient coumaphos concentration for control of fever ticks; however, there has been a need for data on the acaricidal activity of coumaphos in dipping vats to allow for their most economic, yet effective, use. For example, chemical analysis of dipping vats left idle for nearly 2 years indicated that coumaphos was present at about the same concentration calculated for the initial charge; the unanswered question was whether or not the coumaphos was acaricidally active.

Historically, one of the advantages of the use of arsenic, As_2O_3, was the existence of a vat-side test that accurately determined the concentration of arsenic in the vat. The needed concentration could be maintained by addition of water or arsenic as determined by the test. Although a chemical technique has been developed (presented herein) to determine coumaphos concentration in dipping-vat fluids, there have been no data to show that the chemical determination is also an accurate measure of the acaricidal activity of coumaphos. A bioassay technique that measures coumaphos concentration by relating the dilution of a vat sample to the concentration that controls ticks has been needed in order to determine acaricidal activity of the vat contents. If the coumaphos concentration determined by chemical analysis were equal to or at least related to the concentration determined by bioassay, then the chemical analysis could be used as a measure of acaricidal activity. With a rapid chemical analysis it would be necessary to empty, clean, and recharge vats only if warranted—not according to an arbitrary schedule.

The purposes of this study were to determine (1) whether the coumaphos concentration in a dipping vat could be determined by a bioassay technique with engorged *B. microplus* females; (2) the relationship between coumaphos concentration determined by bioassay and concentration determined by chemical analysis; (3) the effect of replenishment of coumaphos and water on biological and chemical concentration; (4) the effect of age and use of a vat on the biological and chemical concentration; (5) the effect of sampling time, either after initial mixing and before dipping, or after cattle had been dipped, on the chemical concentration; and (6) whether the biological concentration of coumaphos is sufficient to kill engorged female cattle ticks.

MATERIALS AND METHODS

Vats

The 10 regularly used vats in the study represented the full range of vat-management conditions and all geographic areas in the Cattle Fever Tick Eradication Program along the Mexico-Texas border. In addition, four disused vats, with coumaphos charges ranging in age from 491 to 742 days were sampled. The vats were managed under the supervision and according to the requirements of the Texas Animal Health Commission and the U.S. Animal and Plant Health Inspection Service, with two modifications in official procedures: the time of required recharging was extended for up to 1 year, and more than 2 animals per gallon of vat capacity were allowed to be dipped.

The 10 regularly used vats were:

Cameron County No. 4 vat.—At Brownsville in Hidalgo County; concrete; 4,430 gallons; water from a canal from the Rio Grande; initially charged on March 31, 1975; test completed on March 26, 1976.

Cotulla vat.—At Cotulla livestock auction in La Salle County; concrete; 6,000 gallons; water from a well; initially charged March 12, 1975; test completed June 9, 1975.

Del Rio vat.—Northwest of Del Rio in Val Verde County; concrete; 4,800 gallons; water from Del Rio

City; initially charged March 6, 1975; test completed March 4, 1976.

El Indio vat.—West of El Indio in Maverick County; concrete; 4,810 gallons; water from a canal from the Rio Grande; initially charged March 3, 1975; test completed March 12, 1976.

Hidalgo No. 75 vat.—At Abram in Hidalgo County; concrete; 4,620 gallons; water from rural water system; initially charged April 29, 1975; test completed March 9, 1976.

Laredo City vat.—Northwest of Laredo in Webb County; concrete; 4,962 gallons; water from Laredo City; initially charged April 30, 1975; test completed October 16, 1975.

Rancho Nuevo vat.—On Guerra Ranch in Starr County; concrete; 3,705 gallons; water from a well; initially charged January 1, 1975, and test completed May 14, 1975; recharged May 28, 1975, and test completed March 15, 1976.

Rancho Viejo vat.—On Briscoe Ranch in Dimmit County; concrete; 4,142 gallons; water from a well; initially charged March 4, 1975, and test completed April 25, 1975; recharged May 9, 1975, and test completed January 14, 1976.

Sandia vat.—Near Falcon in Starr County; concrete; 4,035 gallons; water from a well; initially charged April 9, 1975, and test completed July 30, 1975; recharged August 13, 1975, and test completed March 15, 1976.

Zapata City vat.—West of Zapata in Zapata County; concrete; 3,590 gallons; water from Zapata; initially charged April 14, 1975, and test completed July 29, 1975; recharged August 19, 1975, and test completed March 16, 1976.

The disused vats were:

Ben Bolt vat.—East of Ben Bolt in Jim Wells County; metal; 3,275 gallons; water from Alice; initially charged August 6, 1974.

Midway vat.—Near Alice in Jim Wells County; metal, 3,500 gallons; water from Alice; initially charged March 18, 1974.

Premont vat.—Near Premont in Jim Wells County; concrete; 4,880 gallons; water from Premont; initially charged March 13, 1974.

Saenz vat.—Near Cruz Calle in Duval County; concrete; 3,100 gallons; water from San Diego; initially charged March 22, 1974.

Sample Collection

Samples were taken from each regularly used vat at 2-week intervals, if possible, by submersing collection bottles just beneath the surface of the vat liquid, as follows: two 4-ounce samples after vat mixing but before cattle dipping; two 4-ounce samples immediately after cattle dipping; and a 1-pint sample immediately after cattle dipping. The 4-ounce samples were for chemical analysis; one set taken before and after dipping was sent by mail to the Animal and Plant Health Inspection Service's Veterinary Services Diagnostic Laboratory, Beltsville, Md., and the other set was sent by mail to a laboratory at South Plains College, Levelland, Tex. The pint samples were sent by courier to the U.S. Livestock Insects Laboratory, Kerrville, Tex., and then transshipped to the Science and Education Administration's Cattle Fever Tick Research Laboratory, Falcon Heights, Tex., for bioassay. The bioassay samples were kept refrigerated to prevent or delay decomposition during extended storage. Similar samples for bioassay and chemical analysis were taken from the disused vats after vat contents had been mixed thoroughly; no cattle were dipped.

Chemical Analysis

The following ultraviolet method was used to determine the amount of coumaphos in the vat samples:

1. Thoroughly shake sample from dip vat.
2. Pipet 5 milliliters of the sample into a 100-milliliter volumetric flask containing 50 milliliters of methanol. Shake for 10 minutes.
3. Fill the flask to volume with methanol. Invert 30 times.
4. Filter or centrifuge off suspended material.
5. (A) Pipet 5 milliliters of the filtrate from step 4 into a 25-milliliter volumetric flask and dilute to volume with methanol. Invert 30 times.
   (B) Pipet 5 milliliters of the filtrate from step 4 into a 50-milliliter volumetric flask and dilute to volume with methanol. Invert 30 times.
   (C) Pipet 5 milliliters of the filtrate from step 4 into a 100-milliliter volumetric flask and dilute to volume with methanol. Invert 30 times.
6. In a spectrophotometer take absorbance readings at 315 nanometers and 360 nanometers for solutions 5A, 5B, and 5C.
7. Calculate the coumaphos concentration for solutions 5A, 5B, and 5C:

$$\text{Concentration } (\%) = (0.280)(A_{315} - A_{360})B,$$

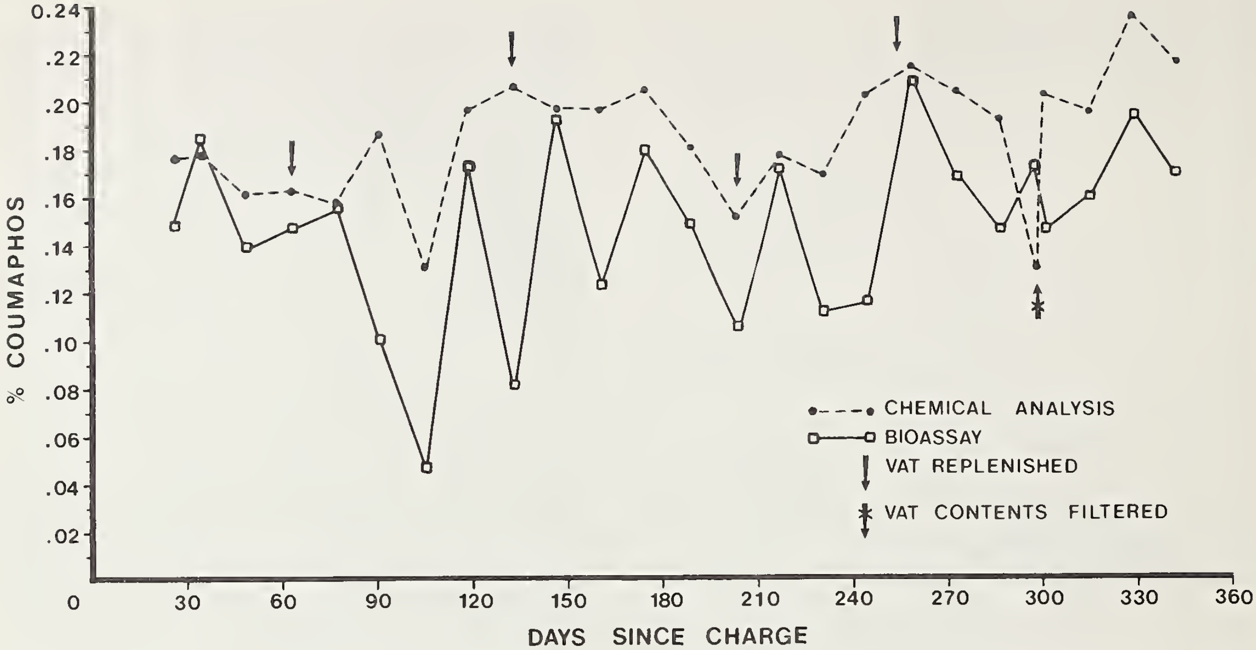

FIGURE 1.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: Cameron County No. 4 vat. See data below.

4,430 gal water + 244 lb coumaphos = 0.165% initial charge

Days since charge	Cumulative No. cattle dipped	Coumaphos (%)		Days since charge	Cumulative No. cattle dipped	Coumaphos (%)	
		Chemical analysis[1]	Bioassay			Chemical analysis[1]	Bioassay
28	244	0.176	0.148	[5]203	1,330	0.150	0.105
35	274	.177	.184	217	1,420	.177	.171
49	338	.162	139	231	—	.169	.110
[2]63	573	.162	.146	245	—	.201	.115
77	613	.158	.155	[6]259	1,559	.213	.208
91	790	.185	.100	273	1,572	.202	.168
105	855	[3].130	.047	287	1,598	[7].190	.145
119	879	.195	.173	[8]298	1,598	[3].129	.172
[4]133	940	.204	.081	301	1,691	[7].200	.143
147	1,037	.196	.192	315	1,749	.194	.159
161	1,084	.196	.123	329	1,805	.234	.193
175	1,185	.202	.178	343	1,871	.213	.168
189	1,289	.180	.147				

[1]After-dipping sample analyzed at Levelland, Tex., unless otherwise noted.
[2]Replenished at 63 days: 950 gal water + 52 lb coumaphos = 0.164%.
[3]Before-dipping sample analyzed at Levelland, Tex.
[4]Replenished at 133 days: 950 gal water + 52 lb coumaphos = 0.164%.
[5]Replenished at 203 days: 870 gal water + 48 lb coumaphos = 0.165%.
[6]Replenished at 254 days: 1,000 gal water + 56 lb coumaphos = 0.168%.
[7]After-dipping sample analyzed at Beltsville, Md.
[8]Sample taken after contents filtered.

where A_{315} = absorbance at 315 nanometers,
A_{360} = absorbance at 360 nanometers,
and $B = 1$ for solution 5A, 2 for solution 5B, and 4 for solution 5C.

The values for the three solutions should agree. However, if the amount of coumaphos is very low, there may be some variation. If variation occurs, then the value for 5C may be discarded. It may be found with experience that only two readings are necessary, and with dip vats containing about 0.25 percent active ingredient, these readings would be 5B and 5C.

Bioassay Procedure

Samples of vat fluids were bioassayed at the Cattle Fever Tick Laboratory by the technique of Drummond et al. (1973, 1976). Engorged female southern cattle ticks (from the standard laboratory colony), allowed to detach naturally from cattle, were placed in groups of 10, weighed, and dipped for 30 seconds in serial dilutions of a freshly prepared standard suspension containing 0.175 percent coumaphos (wettable powder) and in serial dilutions of the dipping-vat samples containing an unknown amount of coumaphos. After dipping, the groups of 10 females were allowed to dry and were then placed in vials in an incubator at 27° C and above 80 percent relative humidity for 2 weeks. After oviposition, the females were discarded, and the eggs were weighed, returned to the same vials, and held at the same conditions for about a month, when the percentage of hatch was estimated visually.

The effectiveness of a dilution was determined by the effect on the estimated reproduction (ER) of the treated females:

$$\mathrm{ER} = \frac{\text{Wt. eggs laid (g)}}{\text{Wt. females (g)}} \times \text{est. hatch (\%)} \times 20{,}000,$$

where 20,000 = the estimated number of larvae in 1 gram of eggs.

The percentage of control afforded by a dilution was determined by comparing the ER of the treated females with that of females collected from cattle at the same time and dipped in an emulsion of xylene and Triton X–100:

$$\text{Control (\%)} = \frac{\text{ER untreated ticks} - \text{ER treated ticks}}{\text{ER untreated ticks}} \times 100.$$

The control given by a serial dilution of freshly prepared coumaphos and a vat sample was analyzed by a log-probit method on a programmable calculator at the U.S. Livestock Insects Laboratory, to arrive at the LC_{50}, LC_{90}, and LC_{99}. To determine the amount of coumaphos in the vat sample, the following formula was used:

$$\text{Coumaphos in sample (\%)} = \frac{0.175\% \times \text{dilution to obtain } LC_{50}}{\text{Dilution to obtain } LC_{50} \text{ in sample}}.$$

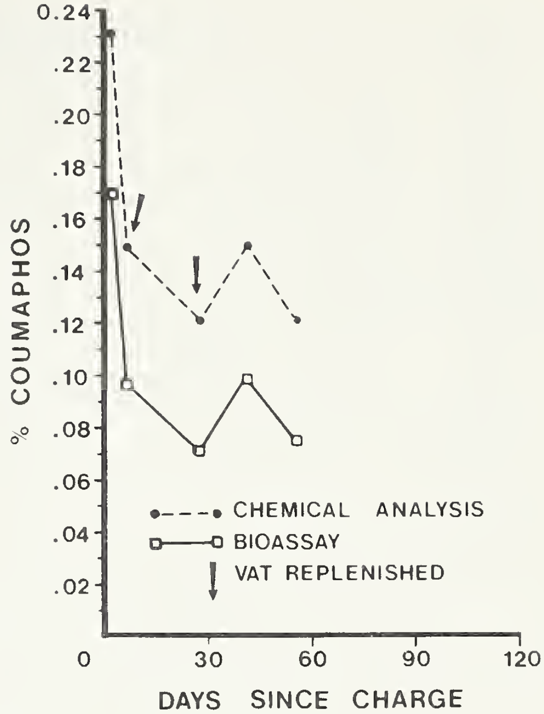

Figure 2.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: Cotulla vat. See data below.

6,000 gal water + 336 lb coumaphos = 0.168% initial charge

Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay
0	0	0.23	0.170
[2]7	50	.15	.096
[3]28	112	.12	.071
42	242	.15	.097
56	328	.12	.075

[1]After-dipping sample analyzed at Beltsville, Md.
[2]Replenished at 7 days: 300 gal water + 16 lb coumaphos = 0.160%.
[3]Replenished at 28 days: 115 gal water from rain + 8 lb coumaphos = 0.20%.

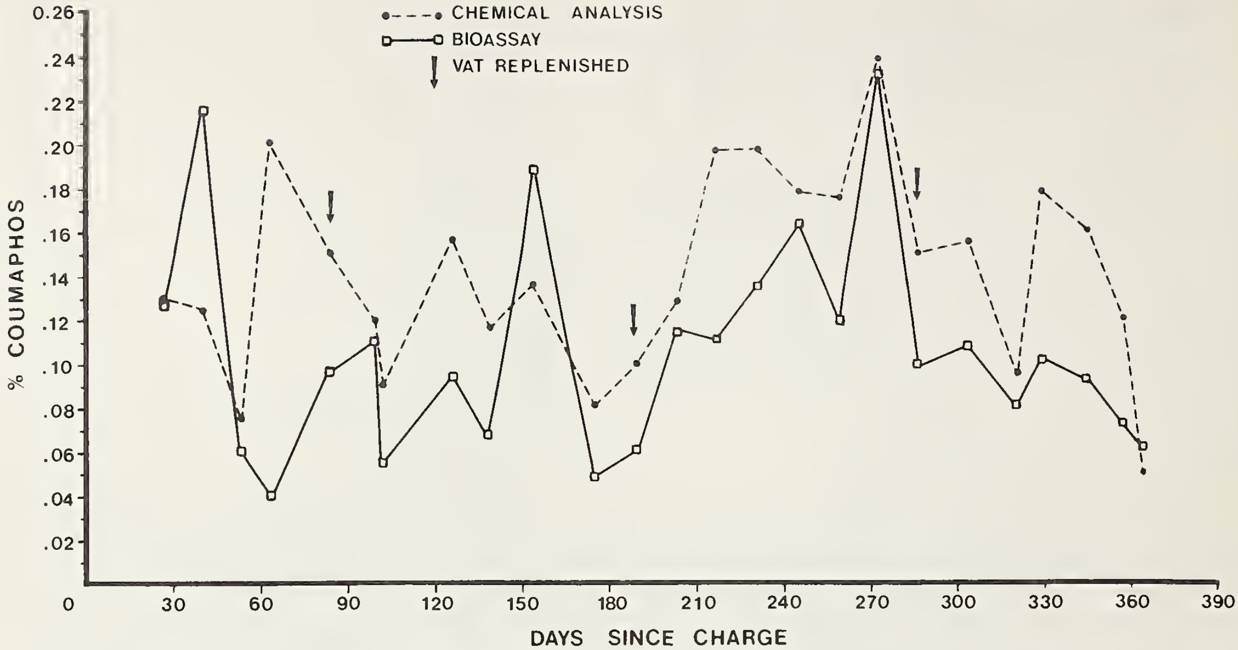

FIGURE 3.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: Del Rio vat. See data below.

4,800 gal water + 264 lb coumaphos = 0.165% initial charge

Days since charge	Cumulative No. cattle dipped	Coumaphos (%)		Days since charge	Cumulative No. cattle dipped	Coumaphos (%)	
		Chemical analysis[1]	Bioassay			Chemical analysis[1]	Bioassay
26	0	[2]0.13	0.127	217	214	[3]0.198	0.112
40	39	[3].124	.216	231	—	[3].198	.136
53	39	[3].076	.060	245	—	[3].178	.163
63	46	[4].20	.040	259	262	[3].177	.119
[5]84	62	[3].151	.096	273	268	[3].239	.231
99	75	[3].119	.110	[7]286	276	[3].150	.099
112	75	[3].090	.054	303	289	[3].156	.107
126	85	.157	.093	320	299	[3].095	.080
139	93	[3].118	.066	329	312	[3].179	.101
154	124	[3].136	.188	344	312	[3].160	[8].092
175	173	[3].081	.049	357	312	[2].120	.073
[6]189	199	.099	.060	364	312	[2].050	[8].062
203	212	[3].127	.115				

[1]After-dipping sample analyzed at Levelland, Tex., unless otherwise noted.
[2]Before-dipping sample analyzed at Beltsville, Md.
[3]Before-dipping sample analyzed at Levelland, Tex.
[4]After-dipping sample analyzed at Beltsville, Md.
[5]Replenished at 84 days: 700 gal water + 38 lb coumaphos = 0.163%.
[6]Replenished at 187 days: 800 gal water + 44 lb coumaphos = 0.165%.
[7]Replenished at 286 days: 800 gal water + 44 lb coumaphos = 0.165%.
[8]Average of 2 determinations.

Statistical Analysis

Whether the concentration of coumaphos determined by bioassay was correlated with the concentration determined by chemical analysis was determined by standard correlation analysis of data from individual vats, from all vats, from the two sampling times, and from the two laboratories. In addition, all the bioassay and chemical data were subjected to regression analysis to determine the statistical relationship between the two sets of data. Because of the completeness of the data and because the chemical and bioassay samples were collected at the same time, usually the coumaphos concentration in the sample collected after dipping and analyzed at Levelland was used for comparison with the concentration determined by bioassay. Whenever that sample was not available, the following samples were used in priority: (1) before-dipping sample analyzed at Levelland, (2) after-dipping sample analyzed at Beltsville, and (3) before-dipping sample analyzed at Beltsville.

RESULTS AND DISCUSSION

Coumaphos Concentration and Activity

With the bioassay technique described above, we were able to determine the biological concentration of coumaphos in the vat samples. The data on dip age, vat use, and coumaphos concentration as determined by bioassay and chemical analysis in the 10 regularly used vats are presented in figures 1–10. There is considerable variation in the time the initial charge stayed in the vat, the frequency of replenishment, and the number of cattle dipped. All vats, except for the Rancho Nuevo vat, were initially charged at about 0.165 percent coumaphos and replenished at the same rate. At Rancho Nuevo, the initial charge was 0.165 percent and replenishment was at 0.24 percent; the second charge was 0.24 percent and replenishment was at 0.165 percent.

Toward the end of the study the contents of several vats—Cameron County No. 4, El Indio, Rancho Nuevo, and Zapata City—were passed through a Hydrosieve filter to remove debris. Although no tests were conducted to determine the effect of filtration on the coumaphos concentration, the data suggest that filtering did not cause a remarkable change.

In general, the amount of coumaphos in the vats remained about 0.165 percent, except for the concentration of the Cameron County No. 4 vat, which increased after about 250 days, and that of the Laredo City vat, which increased after about 100 days. Probably because of the rigid replenishment requirements, the coumaphos concentration was not very low before a vat was replenished. Therefore, the effect of replenishment was also not marked; rather, it appears that replenishment satisfactorily maintained the coumaphos concentration at the initial level.

The relationship between the average amount of coumaphos in the 10 regularly used vats as determined by chemical analysis and bioassay is presented in table 1. The average coumaphos concentration determined by bioassay is about 75 percent of that determined by chemical analysis. There is considerable variation in the significance of the correlation coefficients: four vats and the total sample have highly significant correlation coefficients (probability of $r=0$ of less than 1 chance in 100) and four have significant correlations (probability of $r=0$ of less than 1 chance in 20); the coefficients of the other six are not significant. The small number of samples from some vats made statistical analysis less sensitive than when data were numerous.

To determine whether there was an overall relationship between the coumaphos concentrations by bioassay and by chemical analysis, all 166 observations were used in a regression analysis (fig. 11). There is a highly significant relationship ($r=0.628$). Also, the regression line almost passes through the origin, indicating that when concentration by chemical analysis is zero, the concentration by bioassay is also zero. The standard error of the estimate of the regression equation ($\hat{Y}=0.0085+0.692X$) is ± 0.0368. Therefore, the concentration by bioassay (and thus acaricidal activity) can be statistically determined from the concentration determined by chemical analysis within fairly limited confidence intervals.

Why the coumaphos concentration by bioassay, though consistent, is less than that by chemical analysis is unknown. Possibly, processing during the chemical analysis released coumphos bound up with debris in the sample, and the bioassay technique could not have measured this amount. Also, that the bioassay was not conducted on certain samples until a year or more after collection may be a factor in this relationship, although in several

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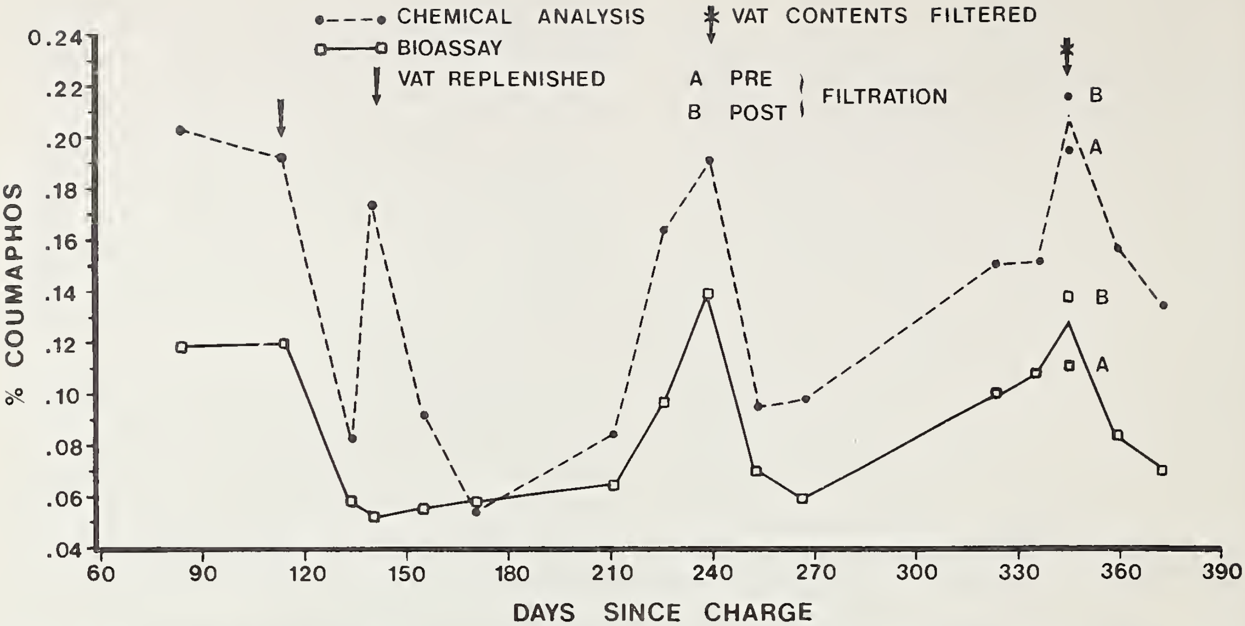

FIGURE 4.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: El Indio vat. See data below.

4,810 gal water + 268 lb coumaphos = 0.167% initial charge

Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay	Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay
85	369	[2]0.200	0.115	254	1,290	[4]0.092	0.065
[3]115	544	[4].119	.116	268	1,290	[4].095	.057
135	544	[5].080	.058	324	1,392	.146	.095
142	544	.170	.050	337	1,410	.147	.103
156	748	[5].090	.053	346	1,634	.190	.107
171	748	[4].053	.055	[6]346	1,634	[4].212	.133
212	748	[4].082	.061	360	1,634	.152	.080
227	1,172	[2].160	.093	375	1,638	.113	.065
240	1,204	.187	.137				

[1]After-dipping sample analyzed at Levelland, Tex., unless otherwise noted.
[2]After-dipping sample analyzed at Beltsville, Md.
[3]Replenished at 115 days: 800 gal water + 44 lb coumaphos = 0.165%.
[4]Before-dipping sample analyzed at Levelland, Tex.
[5]Before-dipping sample analyzed at Beltsville, Md.
[6]Sample taken after vat contents were filtered.

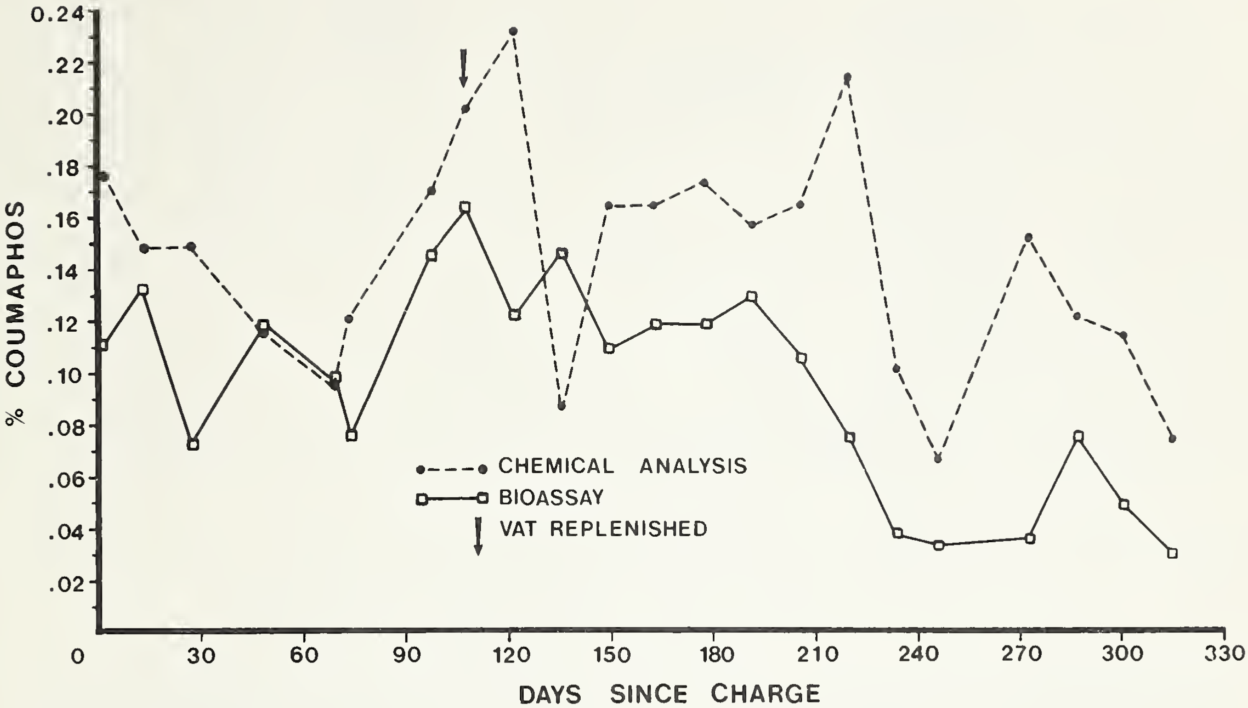

FIGURE 5.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: Hidalgo No. 75 vat. See data below.

4,620 gal water + 254 lb coumaphos = 0.165% initial charge

Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay	Days since charge	Cumulatve No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay
0	38	0.176	0.112	164	345	0.164	0.117
14	104	.147	.132	178	376	.172	.117
28	162	.149	.073	192	404	.157	.127
49	171	.114	.116	206	433	.164	.105
70	181	.094	.098	220	457	.213	.075
84	189	.120	.076	234	482	.100	.037
98	211	.170	.145	246	482	[3].066	.033
[2]108	233	.201	.162	273	486	[3].152	.035
122	267	.232	.122	287	487	[4].120	.075
136	295	.083	.143	301	487	[3].114	[5].047
150	320	.162	.109	315	515	[3].073	[5].029

[1]After-dipping sample analyzed at Levelland, Tex., unless otherwise noted.
[2]Replenished at 108 days: 840 gal water + 48 lb of coumaphos = 0.171%.
[3]Before-dipping sample analyzed at Levelland, Tex.
[4]Before-dipping sample analyzed at Beltsville, Md.
[5]Average of 2 determinations.

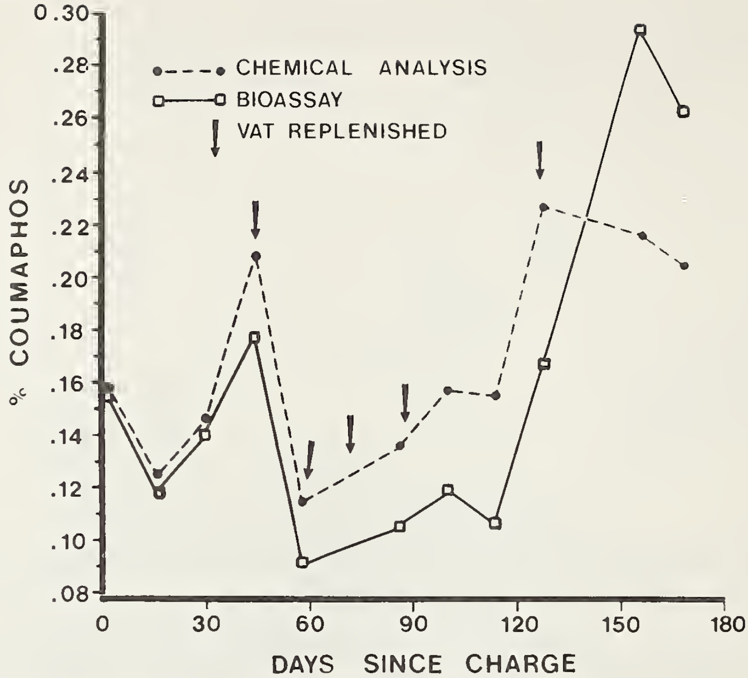

FIGURE 6.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: Laredo City vat. See data below.

4,962 gal water + 276 lb coumaphos = 0.167% initial charge

Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay	Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay
1	27	0.158	0.154	[5]100	3,694	0.157	0.119
16	537	.123	.118	114	4,403	.154	.105
30	614	.144	.140	[6]128	5,285	.224	.168
[2]45	1,005	.208	.177	157	6,067	.215	.292
[3]58	1,371	.114	.091	169	6,191	.202	.260
[4]86	3,293	.136	.106				

[1]After-dipping sample analyzed at Levelland, Tex.
[2]Replenished at 45 days: 1,100 gal water + 60 lb coumaphos = 0.163%.
[3]Replenished at 58 days: 1,100 gal water + 60 lb coumaphos = 0.163%.
[4]Replenished at 72 days: 860 gal water + 48 lb coumaphos = 0.167%.
[5]Replenished at 88 days: 752 gal water + 38 lb coumaphos = 0.151%.
[6]Replenished at 127 days: 855 gal water + 48 lb coumaphos = 0.168%.

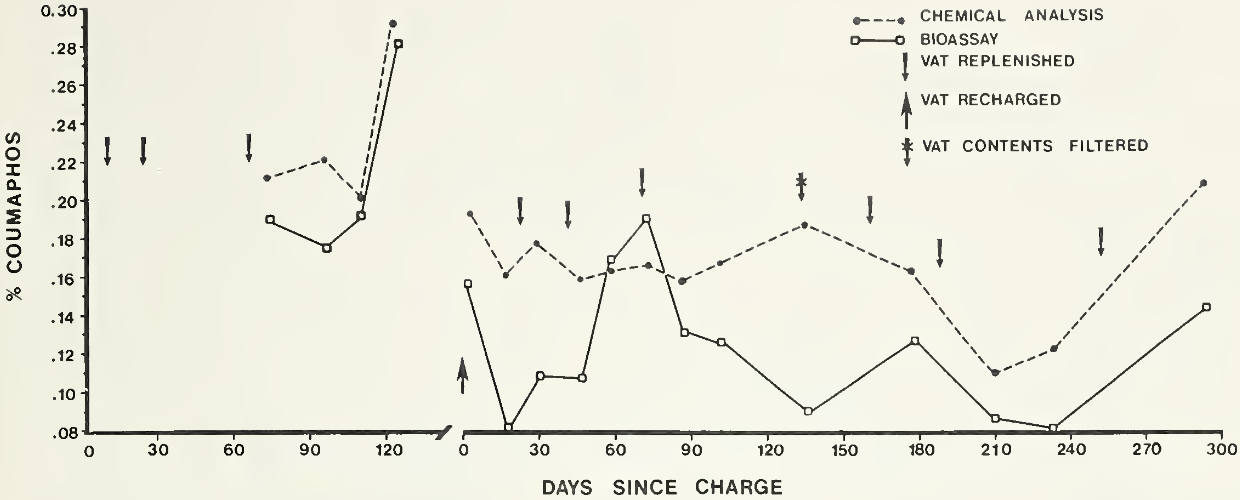

FIGURE 7.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: Rancho Nuevo vat. See data below.

Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay
3,705 gal water + 204 lb coumaphos = 0.165% initial charge			
[2]79	—	[3]0.21	0.188
93	—	[3].22	.173
107	3,145	[3].20	.191
121	3,256	[3].29	.280
3,705 gal water + 296 lb coumaphos = 0.24% recharge			
0	98	0.193	0.156
14	379	.159	.080
[4]28	892	.175	.107
[5]43	1,026	.157	.106
3,705 gal water + 296 lb coumaphos = 0.24% recharge—Continued			
56	1,161	0.162	0.169
[6]70	1,488	.164	.188
84	1,894	.155	.129
98	2,021	.165	.125
[7]132	2,182	.185	.098
[8]175	2,339	.161	.122
[9]208	2,414	.107	.083
231	2,414	[10].120	.078
[11]292	2,795	.204	.141

[1]After-dipping sample analyzed at Levelland, Tex., unless otherwise noted.
[2]Replenished as follows: Day 9: 680 gal water + 54 lb coumaphos = 0.24%. Day 22: 850 gal water + 68 lb coumaphos = 0.24%. Day 65: 800 gal water + 64 lb coumaphos = 0.24%.
[3]After-dipping sample analyzed at Beltsville, Md.
[4]Replenished at 21 days: 580 gal water + 32 lb coumaphos = 0.165%.
[5]Replenished at 40 days: 580 gal water + 32 lb coumaphos = 0.165%.
[6]Replenished at 69 days: 650 gal water + 37 lb coumaphos = 0.170%.
[7]Vat filtered; sample taken before filtering.
[8]Replenished at 159 days: 800 gal water + 44 lb coumaphos = 0.165%.
[9]Replenished at 187 days: 940 gal water + 48 lb coumaphos = 0.153%.
[10]Before-dipping sample analyzed at Levelland, Tex.
[11]Replenished at 250 days: 685 gal water + 40 lb coumaphos = 0.175%.

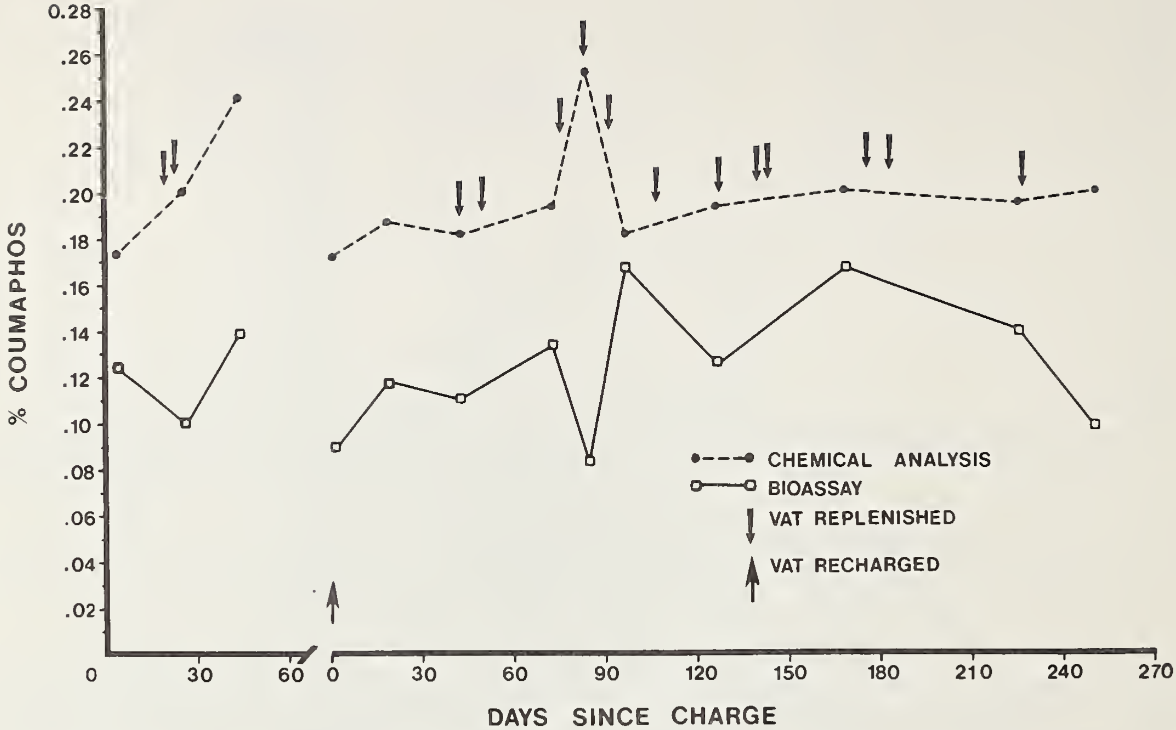

FIGURE 8.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: Rancho Viejo vat. See data below.

Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay	Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay
4,142 gal water + 228 lb coumaphos = 0.165% initial charge				4,142 gal water + 228 lb coumaphos = 0.165% recharge—Continued			
3	141	0.175	0.123	[6]73	1,875	0.192	0.131
[2]25	676	.200	.100	[7]83	2,495	.250	.081
43	966	[3].240	.134	[8]96	2,697	.180	.164
4,142 gal water + 228 lb coumaphos = 0.165% recharge				[9]126	4,347	.193	.124
				[10]168	5,227	.197	.163
0	0	[4]0.170	0.089	[11]225	7,924	.193	.135
18	343	.185	.118	250	9,292	.195	.096
[5]42	899	.179	.109				

[1]After-dipping sample analyzed at Levelland, Tex., unless otherwise noted.
[2]Replenished as follows: Day 17: 500 gal water + 28 lb coumaphos = 0.168%. Day 22: 350 gal water + 20 lb coumaphos = 0.171%.
[3]After-dipping sample analyzed at Beltsville, Md.
[4]Before-dipping sample analyzed at Levelland, Tex.
[5]Replenished at 42 days: 650 gal water + 36 lb coumaphos = 0.166%.
[6]Replenished at 49 days: 500 gal water + 28 lb coumaphos = 0.168%.
[7]Replenished at 76 and 83 days: 500 gal water + 28 lb coumaphos = 0.168%.
[8]Replenished at 91 days: 360 gal water + 20 lb coumaphos = 0.166%.
[9]Replenished at 106 days: 500 gal water + 28 lb coumaphos = 0.168% and replenished at 126 days: 700 gal water + 39 lb coumaphos = 0.167%.
[10]Replenished at 139 days: 638 gal water + 36 lb coumaphos = 0.169% and replenished at 144 days: 905 gal water + 52 lb coumaphos = 0.172%.
[11]Replenished as follows: Day 175: 839 gal water + 48 lb coumaphos = 0.171%. Day 181: 967 gal water + 56 lb coumaphos = 0.173%. Day 225: 433 gal water + 24 lb coumaphos = 0.166%.

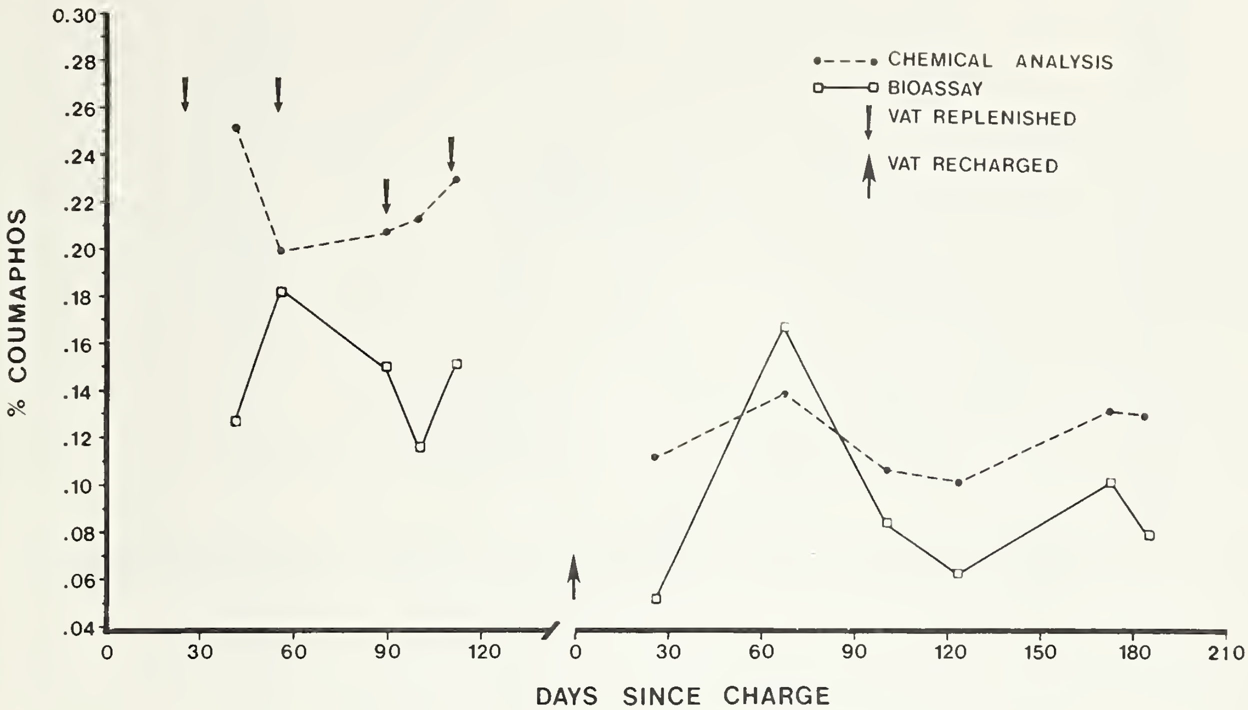

FIGURE 9.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: Sandia vat. See data below.

Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay	Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay
4,035 gal water + 224 lb coumaphos = 0.166% initial charge				4,035 gal water + 222 lb coumaphos = 0.165% recharge			
[2]42	1,467	0.232	0.125	26	837	[6]0.110	0.051
[3]56	1,849	.198	.183	68	837	[7].138	.165
[4]90	2,729	.206	.149	101	837	[7].105	.082
100	3,177	.211	.116	124	837	[6].100	.062
[5]112	3,964	.227	.170	173	837	[6].130	.100
				185	837	[7].128	.074

[1]After-dipping sample analyzed at Levelland, Tex., unless otherwise noted.
[2]Replenished at 28 days: 755 gal water + 42 lb coumaphos = 0.167%.
[3]Replenished at 56 days: 1,000 gal water + 55 lb coumaphos = 0.165%.
[4]Replenished at 90 days: 870 gal water + 25 lb coumaphos = 0.165%.
[5]Replenished at 111 days: 450 gal water + 25 lb coumaphos = 0.165%.
[6]Before-dipping sample analyzed at Beltsville, Md.
[7]Before-dipping sample analyzed at Levelland, Tex.

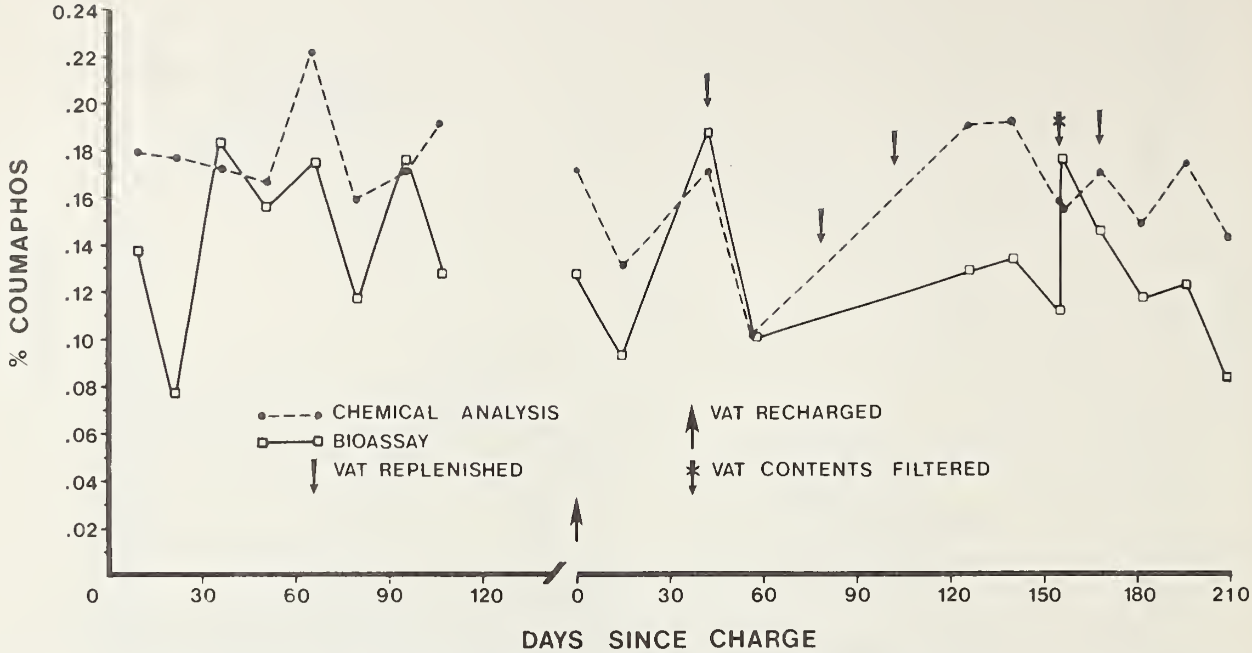

FIGURE 10.—Variation in coumaphos concentration with vat use, as determined by chemical analysis and bioassay: Zapata City vat. See data below.

Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay	Days since charge	Cumulative No. cattle dipped	Coumaphos (%) Chemical analysis[1]	Coumaphos (%) Bioassay
3,590 gal water + 200 lb coumaphos = 0.167% initial charge				3,590 gal water + 196 lb coumaphos = 0.163% recharge			
9	32	0.179	0.138	0	34	0.171	0.126
22	67	.177	.076	15	189	[3].129	.093
36	162	[2].170	.182	[4]42	307	.170	.188
50	210	.166	.155	56	443	.099	.099
65	518	[2].220	.172	[5]126	937	[3].189	.127
79	634	.157	.116	140	989	.191	.131
95	728	[2].170	.172	154	1,148	[3].158	.110
106	873	[2].190	.125	[6]155	1,166	[3].156	.173
				[7]168	1,256	.169	.146
				182	1,301	.146	.116
				196	1,462	[3].172	.121
				210	1,503	.141	.082

[1]After-dipping sample analyzed at Levelland, Tex., unless otherwise noted.
[2]After-dipping sample analyzed at Beltsville, Md.
[3]Before-dipping sample analyzed at Levelland, Tex.
[4]Replenished at 42 days: 570 gal water + 32 lb coumaphos = 0.168%.
[5]Replenished at 78 days: 782 gal water + 44 lb coumaphos = 0.168%, replenished at 112 days: 633 gal water + 36 lb coumaphos = 0.170%.
[6]Sample taken after vat contents were filtered.
[7]Replenished at 168 days: 690 gal water + 36 lb coumaphos + 0.156%.

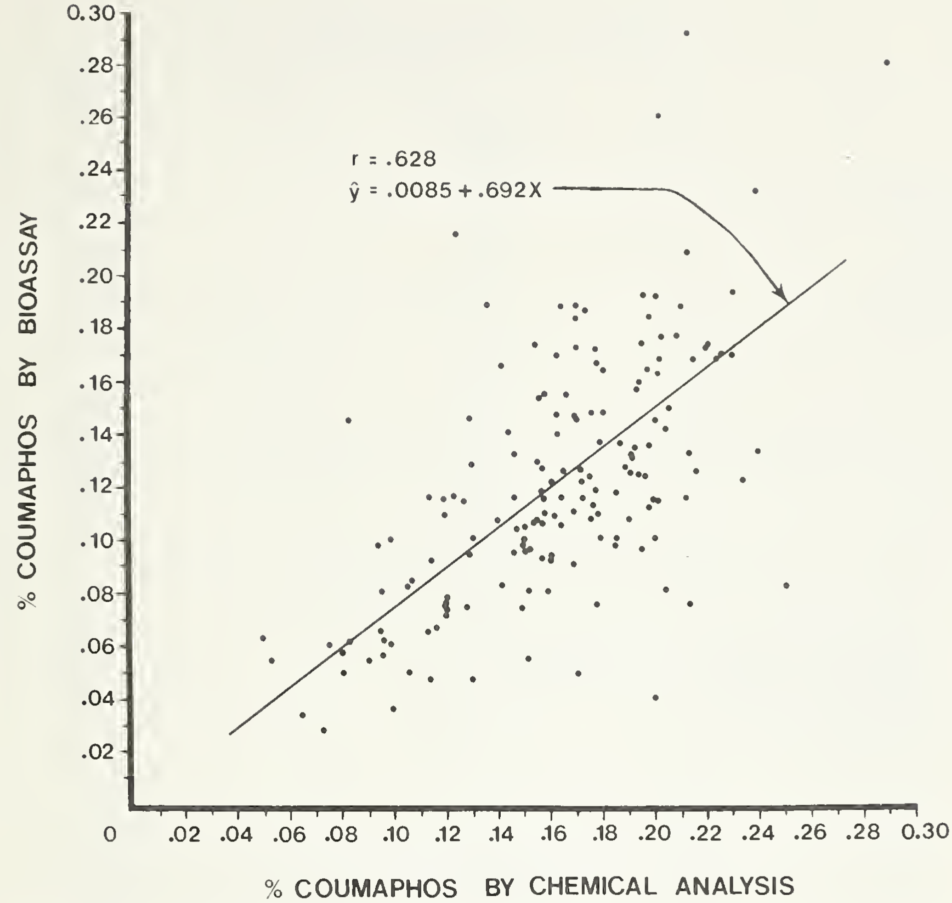

FIGURE 11.—Correlation between chemical analysis and bioassay of 166 vat samples for coumaphos.

Table 1.—Correlation of coumaphos concentrations determined by chemical analysis and bioassay of 10 regularly used vats

Vat	No. samples	Avg. coumaphos conc. (%)		Correlation coefficient (r)
		Chemical analysis[1]	Bioassay	
Cameron No. 4	25	0.184	0.147	0.426*
Cotulla	5	.154	.102	.998**
Del Rio	25	.140	.106	.502*
El Indio	17	.135	.085	.765**
Hidalgo No. 75	22	.143	.095	.502*
Laredo City	11	.167	.157	.785**
Rancho Nuevo	4	.230	.208	.937**
	13	.162	.122	.492[ns]
Rancho Viejo	3	.205	.119	.439[ns]
	10	.193	.121	−.324[ns]
Sandia	5	.215	.149	−.369[ns]
	6	.118	.089	.753*
Zapata City	8	.179	.142	.196[ns]
	12	.158	.126	.497[ns]
Total	166	—	—	—
Average		.161	.120	.628**

[1]After-dipping sample analyzed at Levelland, Tex.
*Significant at 0.05 level.
**Significant at 0.01 level.
[ns]Not significant.

Table 2.—Coumaphos concentrations in four disused vats, as determined by chemical analysis and bioassay

Vat	Vat history		Sample taken (days after charge)	Coumaphos (%)	
	Original coumaphos charge (%)	No. cattle dipped		Chemical analysis[1]	Bioassay
Ben Bolt	0.263	698	491	0.156	0.116
			596	.120	.084
Midway	.164	53	632	.157	.095
			737	.099	.063
Premont	.165	62	637	.252	.195
			742	.179	.131
Saenz	.164	605	628	.340	.269

[1]Sample analyzed at Levelland, Tex.

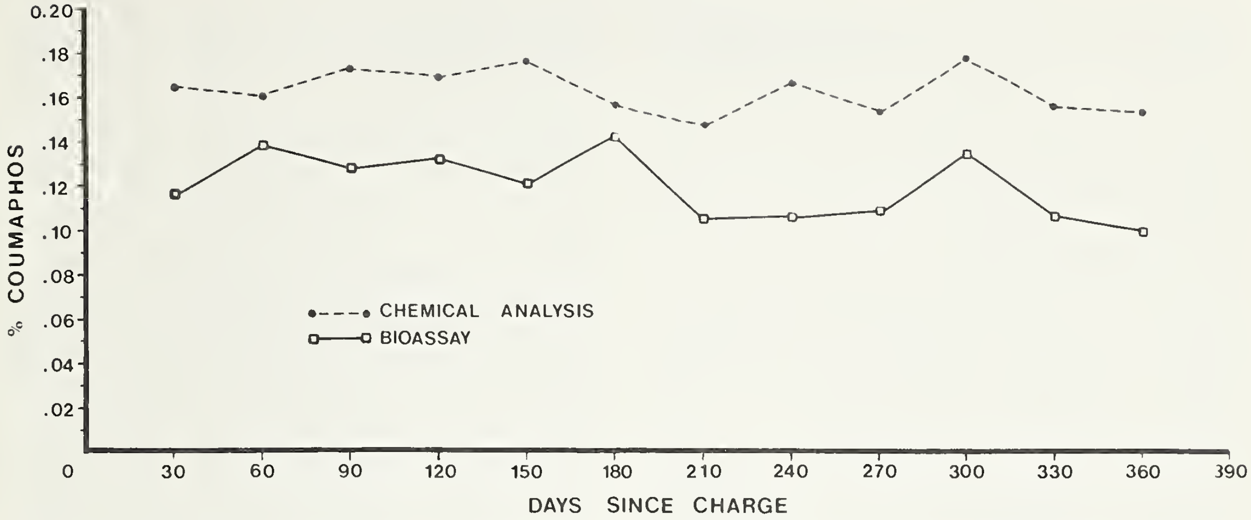

FIGURE 12.—Variation over time of average coumaphos concentration in 10 regularly used vats, as determined by chemical analysis and bioassay. See data below.

Sample taken (days after charge)	No. samples	Avg. coumaphos conc. (%)		Correlaton coefficient (r)
		Chemical analysis[1]	Bioassay	
0–30	23	0.162	0.113	0.513*
31–60	19	.159	.137	.459*
61–90	17	.171	.126	.115[ns]
91–120	19	.168	.129	.756**
121–150	16	.173	.119	.674**
151–180	16	.155	.140	.729**
181–210	11	.143	.103	.823**
211–240	11	.163	.104	.666*
241–270	8	.152	.107	.834**
271–300	8	.173	.133	.673[ns]
301–330	9	.154	.106	.940**
331–360	9	.151	.098	.857**
Total	166	—	—	—
Average		.161	.120	.628**

[1]After-dipping sample analyzed at Levelland, Tex.
*Significant at 0.05 level.
**Significant at 0.01 level.
[ns]Not significant.

samples that were bioassayed twice—often months apart—there is no consistent decrease in acaricidal activity with storage.

When the 166 observations on the 10 regularly used vats are regrouped into categories of 30-day increments after initial charge (fig. 12), the relationship between the chemical analysis and bioassay data is much more significant than when the same data are analyzed by individual vats (table 1). This increase in significance may have been the result of a more equal distribution of numbers of observations at the different time intervals than with the different vats. In general, the average coumaphos concentration in the vats did not decrease with age or use. These data do not agree with those of Schnitzerling and Stone (1968), who reported a highly significant decrease in coumaphos concentration as determined by bioassay of a vat that had been used lightly and fouled artificially. However, their vat was charged with 0.05 percent coumaphos and was not replenished during the study. Palmer (1977) showed that, although

coumaphos concentration in dips artificially polluted with manure was very low, as determined by a bioassay technique with adult *B. microplus*, the coumaphos was still highly acaricidally active against all stages of ticks on cattle dipped in the same vats. He did not determine the relationship between concentration as determined by bioassay and chemical analysis.

The data on the dip age, vat use, and coumaphos concentration in the four disused vats are presented in table 2. The correlation coefficient (r) between coumaphos concentration determined by chemical analysis and by bioassay for all seven samples is 0.995, a highly significant figure. The standard error of the regression equation ($\hat{Y} = -0.024 + 0.861X$) is only ± 0.007. As in the analysis of the regularly used vats, the regression line almost goes through the origin, and there is a small confidence interval. The concentration determined by bioassay is about 73 percent of that by chemical analysis, almost the same relationship for the regularly used vats. Thus, although vats may not be used for 2 years, coumaphos remains acaricidally active, and the chemical analysis is an accurate indication of that activity.

Correlation of Sampling Times and Chemical Analyses

The relationship between coumaphos concentration before and after dipping, as determined at Levelland, Tex., and the relationship between the analyses made at Levelland and Beltsville, Md., of after-dipping samples are summarized in table 3. In the samples analyzed at Levelland, the concentration measured before dipping is, with one exception, less than that after dipping, and there is a significant or highly significant correlation between before and after samples from six of nine vats and for the average data from all vats. The increase in concentration after dipping indicates that additional coumaphos became suspended because of cattle movement in the vats. In general, the concentration determined at Beltsville is greater than that determined at Levelland. Only three of the nine analyses and the average correlations of all analyses are significant or highly significant, and two of the six that are not significant are negative. Slight variation in analytical technique and timing of sample collection probably accounts for most of the

Table 3.—Correlation of coumaphos concentrations determined by chemical analysis of nine regularly used vats, by sampling time and laboratory

Vat	Analyzed at Levelland, Tex., before and after dipping[1]				Analyzed at Levelland, Tex., and Beltsville, Md., after dipping[2]			
	No. samples	Avg. coumaphos conc. (%)		Correlation coefficient (r)	No. samples	Avg. coumaphos conc. (%)		Correlation coefficient (r)
		Before	After			Beltsville	Levelland	
Cameron No. 4	24	0.178	0.189	0.837**	18	0.189	0.190	0.275[ns]
Del Rio	4	.098	.114	.879[ns]	3	.173	.119	.512[ns]
El Indio	13	.123	.153	.637*	12	.161	.152	.680*
Hidalgo No. 75	17	.106	.154	.535*	13	.161	.156	.592*
Laredo City	13	.160	.170	.839**	8	.200	.151	−.339[ns]
Rancho Nuevo	14	.149	.178	.697**	12	.192	.182	.570[ns]
Rancho Viejo	14	.121	.192	.662**	8	.202	.191	−.642[ns]
Sandia	7	.172	.208	.336[ns]	4	.257	.216	.782[ns]
Zapata City	20	.165	.165	.305[ns]	19	.172	.165	.759*
Total	126	—	—	—	97	—	—	—
Average		.146	.172	.596**	—	.183	.170	.559**

[1]Regression equation: $\hat{Y} = 0.100 + 0.494X$.
[2]Regression equation: $\hat{Y} = 0.076 + 0.517X$.
*Significant at 0.05 level.
**Significant at 0.01 level.
[ns]Not significant.

disparity in concentrations between the two laboratories; coumaphos wettable powder begins to settle fairly rapidly, and the samples were taken from the top of the vats, where the greatest variation in concentration would occur in the shortest time.

Adequacy of Vat Charges

Although samples were received at the Falcon Heights, Tex., laboratory starting in March 1975, problems with tick colonies, application and holding techniques, and other factors delayed many bioassays until 1976 or 1977. In the interim the samples were held in a refrigerator to reduce or delay the effect that age and storage might have on coumaphos concentration. Batches of ticks for the bioassay tests were collected periodically, and the data on all ticks collected and dipped in the standard coumaphos dilutions during a distinct period were gathered for a single probit analysis. The variation in the susceptibility of *B. microplus* to coumaphos (25-percent wettable powder) ranges from an LC_{50} of 0.0073 percent to an LC_{50} of 0.0128 percent (table 4). This variation does not appear to be related to season of the year and is not excessive in terms of biological variation. Drummond et al. (1973) listed an LC_{50} value of 0.0142 percent and an LC_{90} value of 0.0317 percent—slightly higher than the figures in table 4. Drummond et al. (1973) also listed an LC_{50} value of 0.0319 percent and an LC_{90} value of 0.0662 percent for *B. annulatus*. Using the same data, we calculate that the LC_{99} for *B. annulatus* is 0.120 percent. Comparison of the coumaphos concentrations determined by bioassay (figs. 1–10) and probit analysis for *B. microplus* (table 4) show that the vat concentrations were equal to or up to 13 times greater than the LC_{99} (the amount calculated to kill 99 percent of the ticks). The averages are: vat = 0.120 percent; LC_{99} = 0.03 percent (a fourfold margin). For *B. annulatus*, the average biological concentration in the vats, 0.120 percent, equals that of the LC_{99}.

Table 4.—Variation in log-probit analysis of bioassay for coumaphos (25% wettable powder), using engorged *B. microplus* females

Date	Slope (b)	Coumaphos (%)		
		LC_{50}	LC_{90}	LC_{99}
1975				
September	3.04	0.0086	0.0227	0.0501
November	5.50	.0085	.0146	.0226
1976				
February	6.66	.0111	.0173	.0248
April	4.55	.0093	.0178	.0301
May	5.02	.0106	.0190	.0307
June	4.04	.0086	.0178	.0323
August	5.51	.0104	.0177	.0274
October	5.20	.0110	.0194	.0308
November	3.79	.0073	.0160	.0302
December	4.75	.0083	.0154	.0256
1977				
May	4.59	.0082	.0156	.0264
June	4.69	.0092	.0172	.0288
July	5.17	.0086	.0153	.0243
August	4.73	.0080	.0150	.0250
September	3.39	.0096	.0230	.0468
October	3.50	.0128	.0298	.0592
December	4.82	.0086	.0158	.0260
1978				
January	4.61	.0128	.0243	.0410
Average	4.65	.0095	.0185	.0323

CONCLUSIONS

1. The bioassay technique with engorged female *B. microplus* is adequate to estimate coumaphos concentration in a dipping vat.

2. There is a significant correlation between coumaphos concentration as determined by chemical analysis and by bioassay with *B. microplus*, although the values by bioassay were about 75 percent of those determined by chemical analysis.

3. When regularly used vats are replenished as mandated by official policy, the length of time that coumaphos is acaricidally active extends through 1 year.

4. In disused vats, there is no loss in acaricidal activity of coumaphos for at least two years after initial charge, and chemical analysis can accurately measure that activity.

5. The relationship between coumaphos concentration in samples taken after vat agitation and before dipping cattle and the concentration in samples taken after dipping indicate that there is considerable variation in effectiveness of mechanical agitation on the mixing of vat contents.

6. The amount of coumaphos (25-percent wettable powder) mandated for initial charge and replenishment (0.165 percent, or 5.5 pounds per 100 gallons of water) is sufficient to kill susceptible, engorged *B. microplus* and *B. annulatus* females because the concentration equals or exceeds the calculated LC_{99} for the ticks.

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